Bioreactor culture duration of engineered constructs influences bone formation by mesenchymal stem cells.

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Public Summary:
Mesenchymal stem cells (MSCs) are a promising cell source for use in bone tissue engineering due to their capacity to differentiate toward bone-forming cells, secrete bioactive factors that stimulate blood vessel ingrowth in vivo, and responsive to various stimuli to instruct cell fate. The physical properties of biomaterials, such as composition, porosity, and stiffness, are potent cues to drive MSCs toward the bone-forming phenotype. In addition, the exposure of cells to fluid flow once on 3-dimensional scaffolds, made possible by perfusion culture in bioreactors, provides another instructive option. Perfusion culture of MSCs seeded in biomaterial scaffolds provides nutrients for cell survival, enhances extracellular matrix deposition, and increases osteogenic cell differentiation. However, there is no consensus on the appropriate perfusion duration of cellular constructs in vitro to boost their bone forming capacity in vivo. We investigated this phenomenon by culturing human MSCs in macroporous composite scaffolds in a direct perfusion bioreactor and compared their response to scaffolds in continuous dynamic culture conditions on an XYZ shaker. Cell seeding in continuous perfusion bioreactors resulted in more uniform cell distribution than static seeding. We observed similar calcium deposition, the functional output of MSC osteogenic differentiation, in all composite scaffolds over 21 days of bioreactor culture, regardless of pore size. Compared to scaffolds in dynamic culture, perfused scaffolds exhibited increased DNA content and expression of osteogenic markers up to 14 days in culture that plateaued thereafter. We then evaluated the effect of perfusion culture duration on bone formation when MSC-seeded scaffolds were implanted in subcutaneously in mice. Human MSCs persisted in all scaffolds at 2 weeks in vivo, and we observed increased blood vessel ingrowth in constructs cultured under perfusion for 7 days relative to those cultured for 1 day within each mouse gender, emphasizing the importance of considering gender-related responses to engineered tissues. At 8 weeks post-implantation, we observed greater bone formation and osteoblastic markers in bioreactor constructs cultured for 14 days compared to those cultured for 1 or 7 days, and acellular constructs. Taken together, these data demonstrate that culturing MSCs under perfusion culture for at least 14 days in vitro improves the quantity and quality of bone formation in vivo. This study highlights the need for optimizing in vitro bioreactor culture duration of engineered constructs to achieve the desired level of bone formation.

Scientific Abstract:
Perfusion culture of mesenchymal stem cells (MSCs) seeded in biomaterial scaffolds provides nutrients for cell survival, enhances extracellular matrix deposition, and increases osteogenic cell differentiation. However, there is no consensus on the appropriate perfusion duration of cellular constructs in vitro to boost their bone forming capacity in vivo. We investigated this phenomenon by culturing human MSCs in macroporous composite scaffolds in a direct perfusion bioreactor and compared their response to scaffolds in continuous dynamic culture conditions on an XYZ shaker. Cell seeding in continuous perfusion bioreactors resulted in more uniform MSC distribution than static seeding. We observed similar calcium deposition in all composite scaffolds over 21 days of bioreactor culture, regardless of pore size. Compared to scaffolds in dynamic culture, perfused scaffolds exhibited increased DNA content and expression of osteogenic markers up to 14 days in culture that plateaued thereafter. We then evaluated the effect of perfusion culture duration on bone formation when MSC-seeded scaffolds were implanted in a murine ectopic site. Human MSCs persisted in all scaffolds at 2 weeks in vivo, and we observed increased neovascularization in constructs cultured under perfusion for 7 days relative to those cultured for 1 day within each gender. At 8 weeks post-implantation, we observed greater bone volume fraction, bone mineral density, tissue ingrowth, collagen density, and osteoblastic markers in bioreactor constructs cultured for 14 days compared to those cultured for 1 or 7 days, and acellular constructs. Taken together, these data demonstrate that culturing MSCs under perfusion culture for at least 14 days in vitro improves the quantity and quality of bone formation in vivo. This study highlights the need for optimizing in vitro bioreactor culture duration of engineered constructs to achieve the desired level of bone formation.

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